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# Recent advances in the medicinal chemistry of group II and group III mGlu receptors†

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Metabotropic glutamate receptors (mGlu receptors) belong to the G-protein-coupled receptors superfamily. They are divided into three groups, in which group II and group III belong to presynaptic receptors that negatively modulate glutamate and γ-aminobutyric acid (GABA) release when activated. In this review, we introduce not only the functions of mGlu receptors, but also the group II and group III allosteric modulators and agonists/antagonists reported over the past five years according to a classification of their structures, with a specific focus on their biological activity and selectivity. In particular, the structure of these compounds and the future directions of ideal candidates are highlighted.



## 1. Introduction

The glutamate system plays a crucial role in the nervous system in the human body. The imbalance distribution of glutamate in the basal ganglia may result in some neurological diseases, such as Parkinson's disease (PD), schizophrenia, depression, and epilepsy.[1] However, the glutamate system is poorly studied compared with dopamine and acetylcholine. Therefore, there is an urgent need to explore the structure and function of glutamate receptors, discover new glutamate receptor modulators, and screen for molecules with better biological activity and fewer side effects.

Glutamate receptors are divided into two groups: ionotropic glutamate receptors (iGlu receptors) and metabotropic glutamate receptors (mGlu receptors). The iGlu receptors are integral membrane proteins composed of four large subunits.[2] According to their sequence similarity, they are classified into three subclasses of receptors: α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor, *N*-methyl-D-aspartate (NMDA) receptor, and kainate receptor.[3] The mGlu receptors belong to the G-protein-coupled receptors superfamily.[4] Based on their sequence homology and ligand-binding profile, the mGlu receptors are separated into three groups.[5–7] Group I ($mGlu_{1/5}$) comprises postsynaptic excitatory receptors that suppress dopamine release when activated.[8] Group II ($mGlu_{2/3}$) is localized in the pre-terminal region of the axon and negatively modulate glutamate and γ-aminobutyric acid (GABA) release.[9–12] The $mGlu_4$ receptor and the $mGlu_7$ receptor are key research areas in group III ($mGlu_{4/6/7/8}$), which are expressed on presynaptic nerve terminals, and inhibit both glutamate and GABA release.[13–15]

As shown in Fig. 1, the mGlu receptors contain a large extracellular N-terminal domain, heptahelical domain, and a C-terminus. Agonists or antagonists can bind to the orthosteric site located in the N-terminal domain to directly activate or inactivate the receptor. However, because of the high sequence homology to the N-terminal domain, agonists/antagonists lack selectivity to different receptors in the same group. Also, most of the agonists/antagonists are glutamate analogs, and can easily cause a desensitization phenomenon. The heptahelical domain, which shares a very low sequence homology, binds with the positive allosteric modulators

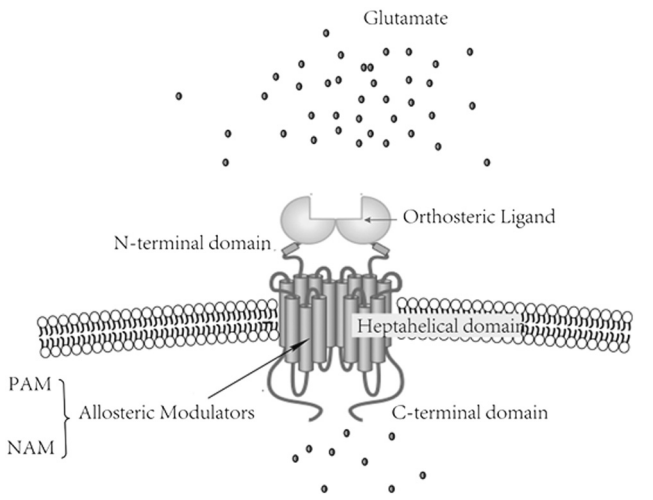


**Fig. 1** The structure of mGlu receptors.

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(PAMs) and negative allosteric modulators (NAMs). PAMs do not activate the receptor directly but enhance the response of the receptor to orthosteric agonists, while NAMs antagonize the activity of agonists in a noncompetitive fashion.[16] Compared with agonists/antagonists, allosteric modulators typically receive more focus.

After the first report on the cloning of a glutamate receptor subunit appeared in 1989,[17] more than a dozen glutamate receptors were cloned in various species in the 1990s. In the late 1990s, the structural biology of iGlu receptors started to be investigated.[18,19] Although two groups[20,21] independently cloned the $mGlu_1$ receptor protein in 1991, a whole family of mGlu receptors was not cloned until 1997. At the beginning of the study on glutamate receptors, researchers focused their attention on the iGlu receptor. However, some scientists discovered that mGlu receptors provided a mechanism by which glutamate could transduce environmental cues and modulate synaptic transmission *via* second messenger signaling pathways compared with iGlu receptors mediating fast responses elicited by glutamate.[22] Thus, mGlu receptors may represent promising targets for a wide range of neurological and psychiatric disorders due to their widespread distribution in the central nervous system (CNS).[16,23]

In the past five years, the research on group II and group III mGlu receptors has made great progress. Fig. 2 exemplifies some approved drugs, candidates, and compounds with excellent potency exampled in this article. Although there are several recent reviews about the $mGlu_1$ receptor and $mGlu_5$ receptor modulators,[24–26] there are no current reviews about group II and group III mGlu receptor modulators and agonists/antagonists. This review thus focuses on new group II and group III modulators and agonists/antagonists reported in the past five years, and analyzes them on the

| Classification | Activity | Disorders | Approved compound |
|---|---|---|---|
| Group Ⅰ | PAM | Schizophrenia | |
| | NAM | Depression, anxiety, addiction | Mavoglurant (Phase Ⅱ/Ⅲ) |
| | | | Basimglurant (Phase Ⅱ) |
| Group Ⅱ | PAM | Schizophrenia, bipolar disorder, addiction | JNJ-40411813 (Phase Ⅱ) |
| | | | AZD-8529 (Phase Ⅱ) |
| | NAM | Depression | Decoglurant (Phase Ⅱ) |
| | Agonist | Addiction, schizophrenia | LY2140023 (Phase Ⅲ) |
| Group Ⅲ | PAM | Cognition, epilepsy, PD | |
| | NAM | Depression, anxiety | |

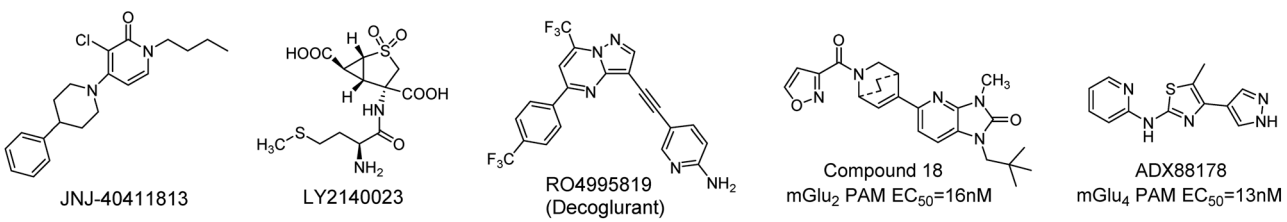



Fig. 2 Some representative drugs to mGlu receptors.

basis of their structures, biological activity, and selectivity. In particular, the structures and the future directions of the ideal candidates are highlighted.

## 2. Group II glutamate receptor allosteric modulators and agonists/antagonists

### 2.1. $mGlu_2$ receptor positive allosteric modulators

**2.1.1. 5-([1,1′-Biphenyl]-3-ylmethoxy)-2,3-dihydro-1*H*-inden-1-one.** In 2005, the Merck company found compound **1** (BINA),[27] but this lacked activity both *in vivo* and *in vitro* for the $mGlu_2$ receptor with unacceptable pharmacokinetic (PK) properties. In 2011, a team from Vanderbilt University[28,29] designed a series of compounds using BINA as the core scaffold. Compound **2** was found to show excellent potency and penetration among these compounds, with an $EC_{50}$ value of 50 nM when tested using a thallium flux assay.[30] It was found that following oral dosing, it could dose-dependently decrease nicotine self-administration in rats. After the structural optimization of compound **2**, some better compounds were found. For example, active compound **3** ($EC_{50}$ = 170 nM) was found to decrease the effects of cocaine self-administration in rats. In particular, compound **4** ($EC_{50}$ = 130 nM) displayed higher activity than compound **3** and expressed excellent PK properties *in vivo* (Fig. 3 and 4).

**2.1.2. *N*-Alkyl-isoquinolone and pyridones.** Trabanco *et al.* designed and synthesized a series of *N*-propyl-5-substituted isoquinolones[31] and *N*-propyl-8-chloro-6-substituted isoquinolones[32] as potent and selective $mGlu_2$ PAMs. Compound **5** was found to be especially effective, with an $EC_{50}$ value of 200 nM using a [$^{35}$S]GTPγS assay,[33] and had a remarkable metabolic stability *in vitro*. Furthermore, compound **6** was identified as the most promising hit from the exploration of 6-substituted chemotypes, with an $EC_{50}$ value of 251 nM. They could also activate the $mGlu_2$ receptor effectively through testing the concentration response curve (CRC) of glutamate on cloned human $mGlu_2$ receptor. The SAR study of 5-substituted isoquinolones indicated that the chloro atom at the C-8 position was not beneficial for potency, even resulting in a significantly low maximal response (Emax). Interestingly, the presence of the 8-chloro atom had a non-significant influence in the 6-substituted isoquinolones.

In fact, *N*-alkyl-isoquinolone derivatives were developed from pyridine derivatives. In 2010, Trabanco and his colleagues[34] started to research the $mGlu_2$ receptor PAM on the basis of the structure of pyridine. They synthesized 58 compounds, and modified at the N-1 and C-5 position mainly. Compound **7** was found to be especially effective, with an $EC_{50}$ value of 525 nM using the [$^{35}$S]GTPγS binging assay. In addition, it significantly attenuated an increase in locomotor activity induced by phencyclidine and displayed good brain levels after IP administration.

However, compound **7** suffered from poor PK properties, which impeded its further development. 1-Isopentyl-3-cyano-4-phenyl-2-pyridone was identified as a hit from a high-throughput screening, and showed good PK properties but displayed low micromolar activity to the $mGlu_2$ receptor. 3D simulation showed the substituent in the C-4 position hindering its combination with the receptor. Hence, 50 compounds were designed and synthesized with an aim to solve this problem. Compound **8**[35] showed good $mGlu_2$ receptor PAM activity *in vitro*, acceptable preliminary safety profile, and good brain exposure, with an $EC_{50}$ value of 361 nM using the [$^{35}$S] GTPγS binging assay. A highly lipophilic substituent in the C-4 position provided compound **8** with acceptable potency and metabolic stability, and its basic pyridine nitrogen had a beneficial effect for its aqueous solubility, while a halogen substituent on the phenyl ring improved its metabolic stability.

In 2014, Trabanco and his colleagues designed and synthesized a series of 4-phenylpiperidine-substituted pyridines as a selective $mGlu_2$ PAM.[36] Compound **9** showed the best potency, with an $EC_{50}$ value of 31 nM using the [$^{35}$S] GTPγS binging assay. However, most of them accompanied the hERG inhibitory activity, hindering their further study. Compound **10** (JNJ-40411813) ($EC_{50}$ = 147 nM) showed good safety margins in various cardiovascular studies and no indication of genotoxicity or cytotoxicity. It was also orally bioavailable and safe. Unfortunately, recent research[37] showed that JNJ-40411813 lacked a strong clinical effect as an adjunctive treatment for patients with major depressive disorder with significant anxious symptoms in the phase IIa clinical study. JNJ-40411813 undoubtedly provided valuable information on its clinical trials, which helped to better assess the potential of the $mGlu_2$ receptor as a target for the treatment of depressive disorder. We are looking forward to the further exploration of JNJ-40411813 in other indications (Fig. 5 and 6).

**2.1.3. Imidazo[1,2-*α*]pyridines and [1,2,4]triazolo[4,3-*α*]pyridine.** Undoubtedly, Trabanco and his colleagues, while working at Johnson & Johnson, made great progress to find a potent and selective $mGlu_2$ receptor PAM. In 2010, they

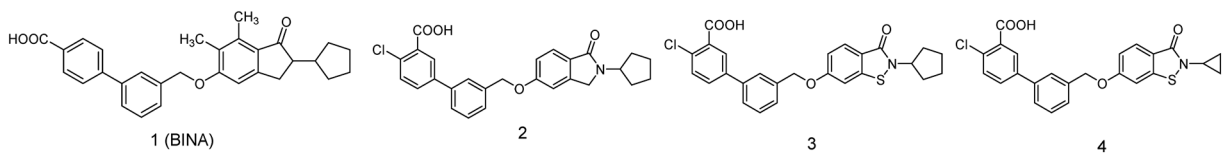



Fig. 3 The structure of $mGlu_2$ receptor PAMs **1**–**4**.

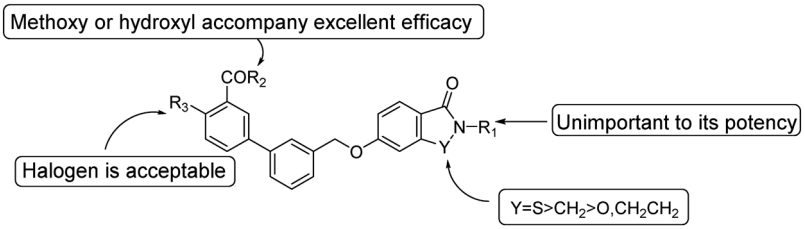



Fig. 4 SAR overview of $mGlu_2$ receptor PAMs **1**–**4**.

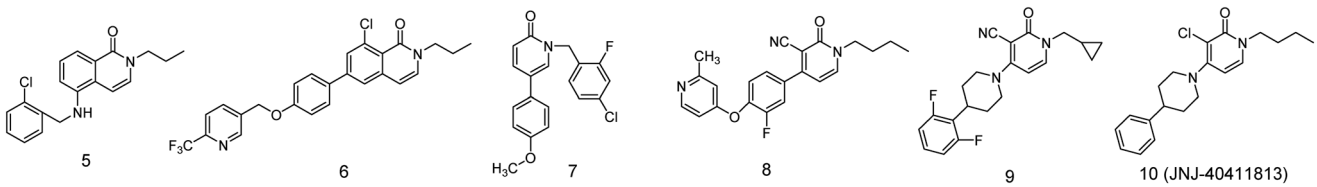



Fig. 5 The structure of $mGlu_2$ receptor PAMs **5**–**10**.

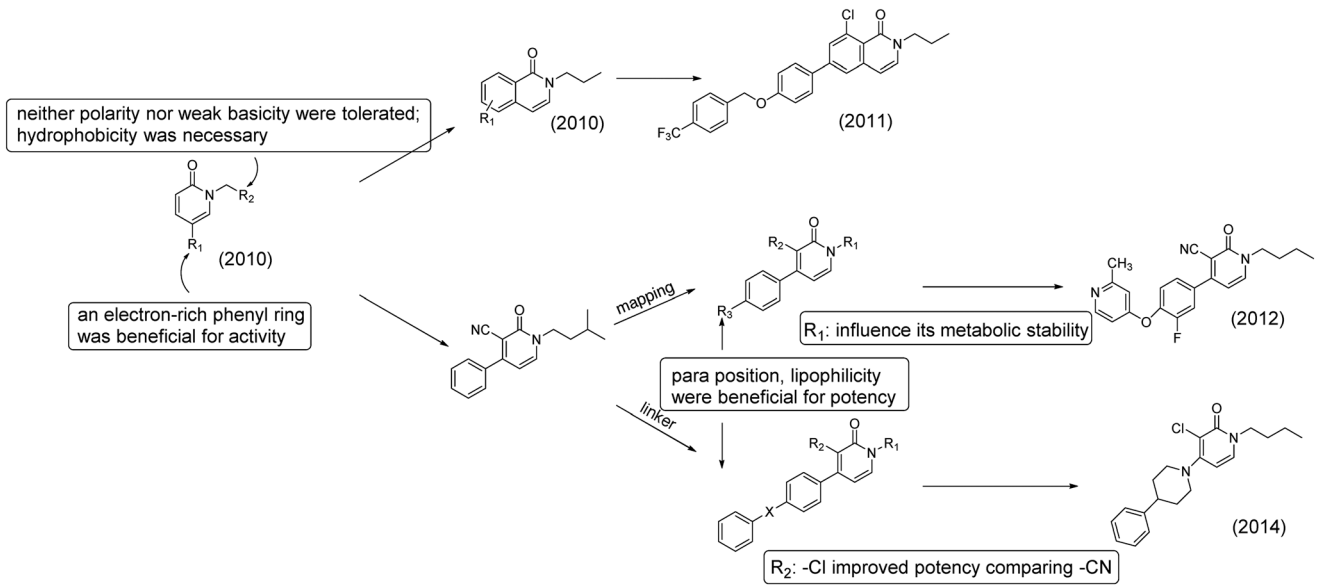



Fig. 6 SAR and strategies overview of $mGlu_2$ receptor PAMs **5**–**10**.

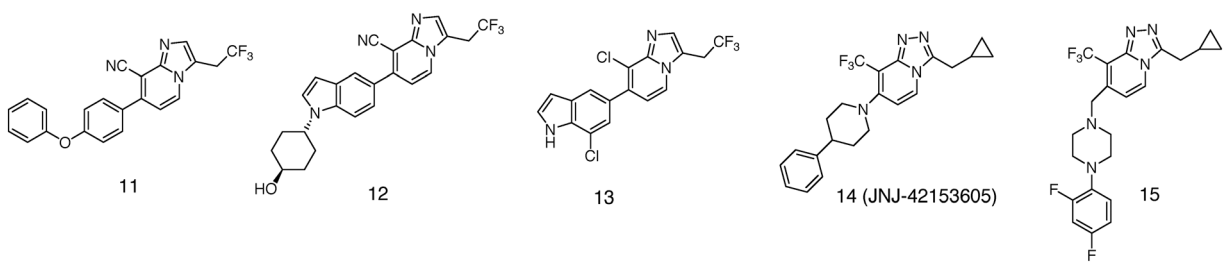



Fig. 7 The structure of $mGlu_2$ receptor PAMs **11**–**15**.

discovered the imidazopyridines using computational techniques based on 3D shape and electrostatic similarity.[38] In this series, compound **11** showed a favorable metabolic stability and selectivity, with an $EC_{50}$ value of 186 nM using the [$^{35}$S]GTPγS binding assay. Comparing the 3D shape of the pyridines and the imidazopyridines (Fig. 8), overall they have

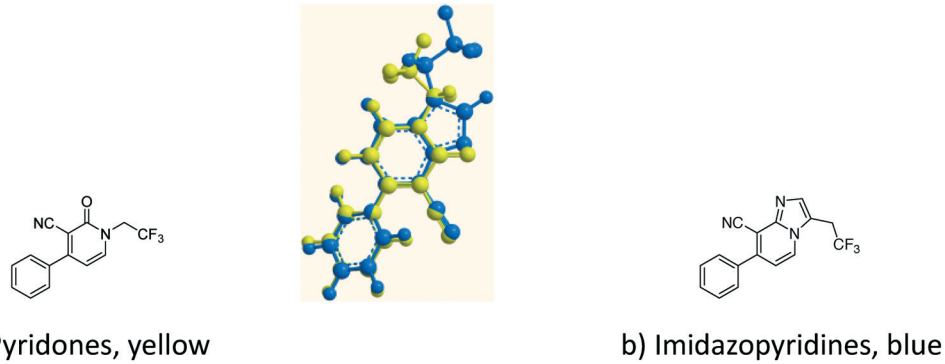



**Fig. 8** Overlapping 3D shape of the pyridines (a) and the imidazopyridines (b).

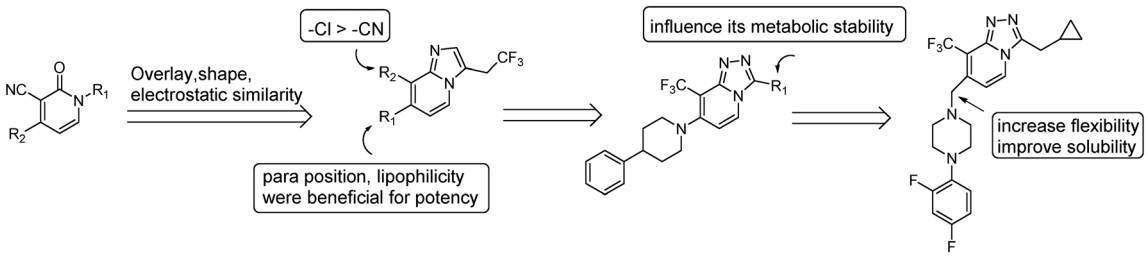



**Fig. 9** SAR and evolution overview of $mGlu_2$ receptor PAMs **11**–**15**.

a similar size, shape, and distribution of substituent groups. SAR studies on the pyridines suitable for the modification in the imidazopyridines are shown in Fig. 9.

However, compound **11** displayed poor oral PK in rats because of its high lipophilicity. Therefore, Trabanco and his colleagues made small modifications at the C-7 and C-8 positions with the aim of decreasing the overall aromaticity and lipophilicity.[39] Compound **12**, with an $EC_{50}$ value of 330 nM using the [$^{35}$S]GTPγS binding assay, was used with an indole group to substitute the phenyl group in the C-7 position, which potentiated its activity and microsomal stability. Then, it was found that the presence of the chloro in the C-8 position was beneficial for brain penetration without being detrimental to other PK parameters. Finally, compound **13** showed excellent ADMET *in vitro* and PK *in vivo* among these compounds, with an $EC_{50}$ value of 85 nM using the [$^{35}$S]GTPγS binding assay.

Initial SAR analysis showed that the trifluoroethyl group was crucial to imidazopyridines' potency and metabolic stability. However, to put it into perspective, exploration was mainly limited to compounds having a trifluoroethyl group, which is one of the few groups to exhibit acceptable potency and metabolic stability. Therefore, they added a nitrogen atom, causing little disruption to the overall molecular shape and features, to reduce its lipophilicity and improve its metabolic stability. This brought about the synthesis and SAR development of novel 1,2,4-trizolopyridines as part of the exploration around pyridines and imidazopyridines. Compound **14** (JNJ-42153605)[40] was found to be the most potent $mGlu_2$ PAM in this series, exhibiting an acceptable PK profile and brain penetration, with an $EC_{50}$ value of 17 nM using the [$^{35}$S]GTPγS binding assay. In 2014, JNJ-42153605 was selected as an excellent candidate to progress toward pharmacological experiments. According to the latest result,[41] JNJ-42153605 had the capacity to dose-dependently and specifically reverse memantine-induced brain activation.

However, compound **14** suffered from poor solubility in water, hindering its further development. In order to improve its aqueous solubility, they placed a methylene spacer between the piperidine ring and the triazole core. Finally, they found a potent, orally bioavailable, soluble and selective triazolopyridine class in 2016. Compound **15** (JNJ-46356479),[42] with an $EC_{50}$ value of 78 nM using the [$^{35}$S]GTPγS binding assay, exhibited a lower clearance, higher oral exposure, and higher bioavailability in rodent and non-rodent species, and was thus considered as an attractive agent to explain the role of selective $mGlu_2$ PAM (Fig. 7).

**2.1.4. Benzimidazoles.** In 2010, Merck's research team found a series of oxazolobenzimidazoles as $mGlu_2$ receptor PAMs. These compounds were evaluated in a fluorescent imaging plate reader (FLIPR) assay that used a Chinese hamster ovary (CHO) cell line coexpressing recombination human $mGlu_2$ receptor and a promiscuous G-protein. In this series, compound **16**[43] exhibited a robust efficacy, with an $EC_{50}$ value of 29 nM. In the pharmacological experiments, compound **16** was shown to have activity in a phencyclidine-induced hyperlocomotion model in rats, providing support for its potential efficacy in treating schizophrenia.

Shortly after, Pfizer's research team designed and synthesized a series of imidazopyridines as $mGlu_2$ receptor PAMs.[44]

These compounds were evaluated in a stable human embryonic kidney cell line expressing the rat $mGlu_2$ receptor, using a FLIPR assay. In this series, compound **17**[44] was a representative lead compound, with an $EC_{50}$ value of 35 nM. It demonstrated the dose-dependent inhibition of mescaline-induced scratching and methamphetamine-induced hyperactivity in mice, providing evidence that it had potential efficacy for the treatment of schizophrenia.

In 2016, on the basis of the early efforts from Merck's labs, Layton and his research team discovered aryl-linked 1,3-dihydro-2*H*-imidazo[4,5-*b*]pyridine-2-ones[45] as a tool molecule to research the $mGlu_2$ receptor. It showed sufficient PK properties and robust activity in preclinical models of psychosis in rats following oral dosing. These compounds were evaluated in a CHO cell line coexpressing recombination human $mGlu_2$ receptor and a promiscuous G-protein, using a FLIPR assay. Compound **18** exerted satisfactory ligand efficiency and lipophilicity, with an $EC_{50}$ value of 600 nM and 213 nM to the human and rat $mGlu_2$ receptors, respectively. Under ADME study *in vivo*, compound **18** revealed acceptable CNS exposure and moderate oral bioavailability. Gratifyingly, compound **18** could fully attenuate hyperlocomotion induced by PCP (appr. 17 000 cm, 0 mg $kg^{-1}$ to less than 2000 cm, 30 mg $kg^{-1}$) or MK-801 (appr. 27 000 cm, 0 mg $kg^{-1}$ to less than 5000 cm, 30 mg $kg^{-1}$) in rats.

In the meanwhile, Merck's research team discovered a novel amino-aza-benzimidazolone structural class as $mGlu_2$ receptor PAMs. Over 70 compounds were synthesized and evaluated in a FLIPR assay using a CHO cell line coexpressing recombinant human $mGlu_2$ receptor and a promiscuous G-protein. Compound **19**[46] was found to be a potent, selective, orally bioavailable $mGlu_2$ PAM, with an $EC_{50}$ value of 16 nM. It showed excellent drug-like properties with robust *in vivo* efficacy in a clinically validated model and was supposed to a candidate drug for the treatment of schizophrenia (Fig. 10).

### 2.2. $mGlu_2$ receptor negative allosteric modulators

In 2015, a research team from Vanderbilt University[47] designed a series of selective $mGlu_2$ receptor NAMs for the intention of evaluating the therapeutic potential of a number of individual targets. Researchers used 1,4-dihydroquinoline-3-carboxamide as a scaffold, and evaluated these compounds with fluorescence-based assays, whereby they measured calcium mobilization induced by receptor activation in a cell line stably expressing either the rat $mGlu_2$ receptor or rat

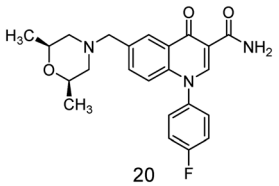



Fig. 11 The structure of $mGlu_2$ receptor NAMs **20**.

$mGlu_3$ receptor along with the promiscuous G-protein. Compound **20** (VU6001192), with an $IC_{50}$ value of 207 nM, showed a favorable profile and highly selectivity to the $mGlu_2$ receptor, and it was considered it would be a useful tool to elucidate the role of the selective inhibition of the $mGlu_2$ receptor (Fig. 11).

### 2.3. $mGlu_3$ receptor negative allosteric modulators

Novel and selective $mGlu_3$ receptor PAMs have not been reported in the past five years. Indeed to date, only four literature reports about $mGlu_3$ receptor NAMs have been reported. The relevant studies though showed that $mGlu_3$ receptor NAMs had potential efficacy in treating depressive disorders.

Utilizing a 'molecular switch' method, Lindsley and co-workers from Vanderbilt University discovered that compound **21** (VU0463597 or ML289)[48] was a novel, CNS-penetrant, and selective $mGlu_3$ receptor NAM. ML289 displayed an $IC_{50}$ value of 660 nM, and furthermore was inactive against the $mGlu_2$ receptor and $mGlu_5$ receptor. According to their exploration, they synthesized 48 compounds, and only found that 4-methoxyphenyl-substituted derivatives displayed the $mGlu_3$ receptor NAM activity. They evaluated these compounds in a calcium assay in which the $mGlu_3$ receptor was co-expressed with the promiscuous G-protein.

In order to understand the biological implications of the selective inhibition of the $mGlu_3$ receptor in the CNS, they continued to explore the structure based on ML289. Compound **22** (ML298)[49] displayed an $IC_{50}$ value of 593 nM using a calcium mobilization assay, and showed favorable DMPK properties, an ancillary pharmacology profile, and good CNS penetration. SAR analysis indicated that the *p*-methoxy moiety was necessary for $mGlu_3$ receptor activity. Also, separation of the isomers showed that the (*R*)-enantiomer was solely responsible for the potency.

In 2015, the latest study about $mGlu_3$ receptor NAMs was reported by Lindsley, Emmitte, and co-workers. They

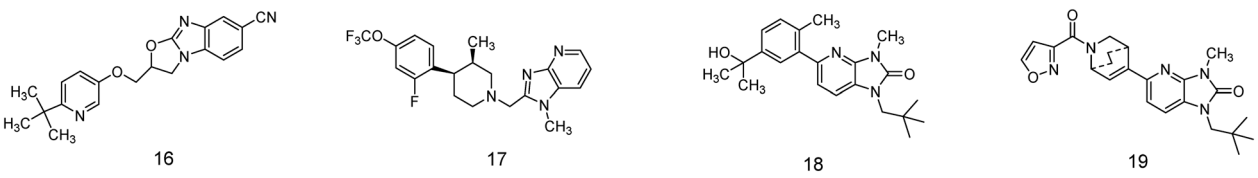



Fig. 10 The structure of $mGlu_2$ receptor PAMs **16**–**19**.

synthesized and explored approximately 100 compounds based on the structure of 5-aryl-6,7-dihydropyrazolo[1,5-*a*]pyrazine-4(5*H*)-ones. Also, they measured these compounds in a fluorescence-based assay, whereby they measured calcium mobilization induced by $mGlu_3$ receptor activation in a cell line stably expressing the rat $mGlu_3$ receptor and the promiscuous G-protein, just as the same for compounds **21** and **22**. An interesting phenomenon was revealed in that a chiral methyl group at the C-7 position of the pyrazine-4(5*H*)-one ring showed a different potency to the $mGlu_3$ receptor. The (*R*)-methyl analog proved highly preferential for $mGlu_3$ receptor NAM activity compared with the (*S*)-methyl analog. In this series, compound **23**[50] displayed an $IC_{50}$ value of 392 nM, with favorable DMPK properties and good bioavailability in rats. Compound **23** was considered to be a valuable tool to understand the biological implications of the selective inhibition of $mGlu_3$ receptor (Fig. 12).

### 2.4. Non-selective $mGlu_{2/3}$ receptor allosteric modulators

In 2014, Cosford and co-workers designed and tested more than 60 carboxylic acid derivatives and found a series of $mGlu_{2/3}$ PAMs displaying excellent potency and efficacy.[51] These analogs were evaluated in thallium flux assays in human embryonic kidney 293 cells expressing heteromeric G-protein-coupled inwardly rectifying potassium 1/2 channels and rat $mGlu_2$ or rat $mGlu_3$ receptors. Among these, compound **24** showed an $EC_{50}$ value of 136 nM to the $mGlu_2$ receptor and an $EC_{50}$ value of 300 nM to the $mGlu_3$ receptor, and dose-dependently decreased cocaine self-administration in rats after a single intraperitoneal dose. In particular, compound **25** displayed agonism-PAM activity toward the $mGlu_2$ receptor and PAM activity at the $mGlu_3$ receptor, with an $EC_{50}$ value of 40 nM to the $mGlu_2$ receptor and an $IC_{50}$ value of 614 nM to the $mGlu_3$ receptor.

A research team from Vanderbilt University designed and synthesized a series of substituted pyrazolo[1,5-*a*]quinazolines as dual $mGlu_2$/$mGlu_3$ NAMs. They synthesized approximately 47 compounds and evaluated them in a calcium mobilization assay. Compound **26**[52] was the most potent inhibitor of group II mGlu receptors discovered from this scaffold, with an $IC_{50}$ value of 245 nM to the $mGlu_2$ receptor and an $IC_{50}$ value of 78 nM to the $mGlu_3$ receptor (Fig. 13).

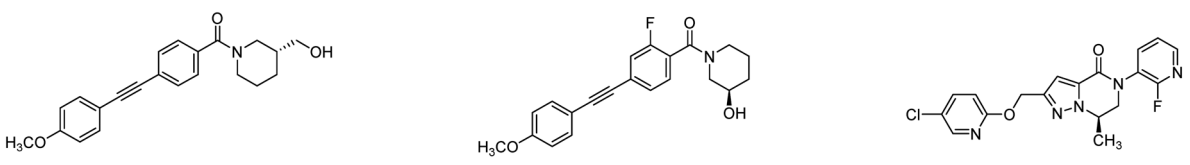
### 2.5. Non-selective $mGlu_{2/3}$ receptor agonists/antagonists

In 2013, Eli Lilly company found a series of 1*S*,2*S*,5*R*,6*S*-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate analogs exhibiting high activity to $mGlu_{2/3}$ receptors and excellent selectivity over the other six mGlu receptors. They found that some substituent groups at the C-4 position showed different influences to the $mGlu_{2/3}$ receptor functional responses. Thus, they synthesized approximately 17 compounds, and performed X-ray diffraction analysis and pharmacological experiments on these to further explore the SAR of small groups substituting at the C-4 position. They evaluated these compounds in inhibiting a forskolin-stimulated cAMP assay for functional responses in human $mGlu_{2/3}$ receptor-expressing cells. Compound **27** (LY459477)[53] displayed an $EC_{50}$ value of 0.56 nM to the $mGlu_2$ receptor and an $EC_{50}$ value of 0.24 nM to the $mGlu_3$ receptor, and showed an ideal bioavailability in rats and potent antipsychotic-like effects in a rodent model of psychosis at certain doses.

During the course of their research, they found that compound **28** showed unprecedented activity as an $mGlu_2$ agonist/$mGlu_3$ antagonist. This puzzling phenomenon inspired their interest, and they continued to optimize these molecules using a small spirocyclic ring at the C-4 position. According to their results, some molecules were found to retain the unexpected $mGlu_2$ agonist/$mGlu_3$ antagonist functions, although with a significant loss in their potency. Among these compounds, compound **29** (LY2934747)[54] displayed potency in models of psychosis and pain, with an $EC_{50}$ value of 8 nM to the $mGlu_2$ receptor and an $EC_{50}$ value of 62 nM to the $mGlu_3$ receptor.

In 2015, substituting thiotriazoles at the C-4 position, they synthesized 14 compounds and found a series of selective and potent human $mGlu_2$ receptor agonists. Compound **30** (LY2812223)[55] displayed a favorable PK behavior in rat models of psychosis, with an $EC_{50}$ value of 6 nM to the human $mGlu_2$ receptor using the inhibition of forskolin-stimulated cAMP assay. The cocrystallization of compound **30** revealed that it had a specific protein–ligand interaction with the $mGlu_2$ receptor, proving the validity of the previous structure–activity analysis.

It was reported that substitution of a benzyl group at the C-3 position caused a functional switch to moderately potent antagonists.[56] This phenomenon came into Eli Lilly's horizon and inspired their interest. In 2016, they synthesized approximately 30 new compounds based on 1*S*,2*R*,4*R*,5*R*,6*S*-2-amino-4-hydroxy bicyclo[3.1.0]hexane-2,6-dicarboxylic acid. Among these, compounds **31**[57] ($hmGlu_2$ $IC_{50}$ = 16 nM, $hmGlu_3$ $IC_{50}$ = 6 nM) and **32**[58] ($hmGlu_2$ $IC_{50}$ = 46 nM, $hmGlu_3$ $IC_{50}$ = 46 nM) showed antidepressant-like activity in the mouse forced-swim test assay, which represented a new tool




21 (VU0463597 or ML289) 22 (ML337) 23

**Fig. 12** The structure of $mGlu_3$ receptor NAMs **21**–**23**.

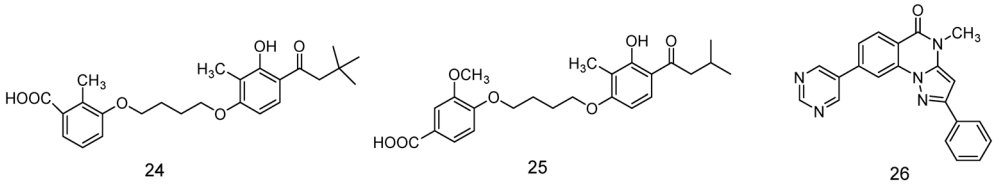



Fig. 13 The structure of $mGlu_{2/3}$ receptor modulators **24**–**26**.



for studying the effects of $mGlu_{2/3}$ antagonism *in vitro* and *in vivo*.

Their research not only found a series of novel and potent $mGlu_{2/3}$ receptor agonists, but also revealed the interactions between receptors and ligands. X-ray crystal structure revealed that these compounds produced hydrogen-binding interactions with the $mGlu_{2/3}$ receptor amino terminal domain. Taking compound **27** as a sample, the C-2 carboxyl group interacted with Arg-64, while the C-4 substituent group interacted with Ser-278 and Arg-64. This is the reason why the substituent group at the C-4 position plays a crucial role in its effect. They realized that a methyl group at the C-$4_\alpha$ position (compound **28**) provided a hydrogen atom in closer contact with the amino acid residue of the $mGlu_3$ receptor and effectively impeded closure of the lobes in the protein, leading to the observed antagonist activity. Through further research, they found that whatever the agonist or antagonist, all were bound to a common hinge to stabilize distinct amino terminal domain conformations (closed for agonists, opened for antagonists). Their analysis revealed the difference between agonists and antagonists on the molecular level, and provided a good reference for other research teams (Fig. 14).

## 3. Group III glutamate receptor allosteric modulators and agonists/antagonists

### 3.1. $mGlu_4$ receptor positive allosteric modulators

$mGlu_4$ receptor PAMs have potential therapeutic uses in schizophrenia, epilepsy, and Parkinson's diseases. In 2003, PHCCC, the first selective $mGlu_4$ receptor PAM, was found by Flor and co-workers. Since then, many tool compounds for the $mGlu_4$ subtype have been discovered.

**3.1.1. Phenyl sulfonamides.** In 2010, Hopkins and coworkers from Vanderbilt University reported a series of novel and selective $mGlu_4$ receptor PAMs identified through functional high-throughput screening method. Using phenyl sulfonamide as a core scaffold, they synthesized 28 compounds and tested them in a calcium mobilization assay. In this series, compound **33**[59] was the most potent $mGlu_4$ receptor PAM, with an $EC_{50}$ value of 20 nM. Unfortunately, these compounds expressed poor PK properties *in vivo*, which prevented their further study.

In order to improve their PK properties, Hopkins and coworkers substituted an amide bond for sulfonamide.[60] They synthesized approximately 30 compounds and utilized two pharmacological assays to test their potency: (1) a calcium mobilization assay and (2) human embryonic kidney cells expressing rat $mGlu_4$ in conjunction with G-protein regulated inwardly rectifying potassium channels to induce thallium flux. In this series, compound **34** exhibited a submicromolar $EC_{50}$ value both in the human and rat $mGlu_4$ receptors, with a human $EC_{50}$ value of 99 nM and a rat $EC_{50}$ value of 106 nM. Unfortunately, it possessed poor metabolic stability because a methoxy group in C-3 position was labile and changeable. In addition, compound **35** (VU0366067) exhibited slightly weaker potency, moderate stability in rat liver microsomes, acceptable PK parameters *in vitro*, and good exposure in both brain and plasma, with an $hEC_{50}$ value of 517 nM and an $rEC_{50}$ value of 570 nM to the $mGlu_4$ receptor.

Subsequently, by substituting phthalimide and sulfimide, they designed and synthesized approximately 30 compounds with excellent PK properties, activity, and selectivity to the $mGlu_4$ receptor. Compound **36**[61] displayed a human $EC_{50}$ value of 136 nM using a calcium mobilization assay and showed selectivity against other mGlu receptors. But, according to the pharmacokinetic experiments in rats, its penetration extent into the brain was poor following intraperitoneal administration because of its molecular weight and polar surface area. Thus, they decreased the molecular weight and polar surface area utilizing phthalimide. This class of compounds displayed enhanced potency, oral bioavailability, and excellent brain exposure compared with sulfimides. Finally, compound **37** (VU0400195, ML182) was found to be orally active in the haloperidol-induced catalepsy model, an

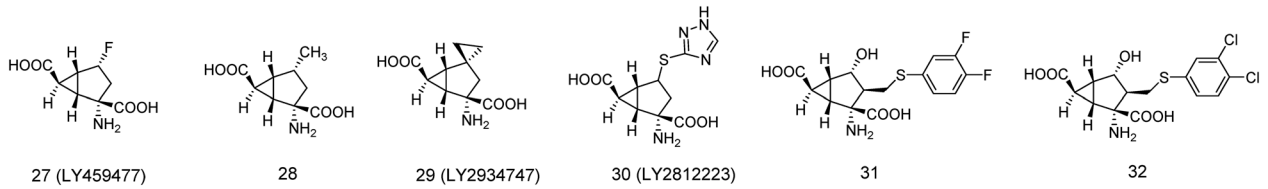



Fig. 14 The structure of $mGlu_{2/3}$ receptor agonists/antagonists **27**–**32**.

anti-parkinsonian model, with a human $EC_{50}$ value of 291 nM and a rat $EC_{50}$ value of 376 nM. They considered that maybe compound **37** would be a useful tool compound for the treatment of Parkinson's disease.

Recently, they replaced the phenyl group centered on a five-membered heterocyclic group and found sulfone analogs as a potent PAM of the $mGlu_4$ receptor. At first, they used a thiazole ring replacing the internal phenyl ring and found compound **38** ($EC_{50}$ = 83 nM)[62] was the most potent compound discovered to date. However, these compounds suffered from poor brain penetration and metabolic instability, which limited their further research. Next, they turned their attention to pyrrole and pyrazole compounds. Although they showed submicromolar $EC_{50}$s to the $mGlu_4$ receptors, they suffered from the same metabolic stability issues as the thiazole compounds. The reason for this was that the benzylic methylene group was oxidized easily. Finally, they eliminated the benzylic site and discovered a series of phenyl sulfonamides. Compound **39**, with an $EC_{50}$ value of 62 nM using a calcium mobilization assay, was the most active molecule among them. Unfortunately, these compounds were not orally bioavailable and showed poor exposure after PO dosing (Fig. 15 and 16).

**3.1.2. Tricyclic thiazolopyrazoles.** In 2010, several patents[63–66] about $mGlu_4$ receptor PAMs were reported by Addex Pharmaceuticals. They found that compound **40**, the thiazolopyrazole derivative, displayed an $EC_{50}$ value of 410 nM tested with a calcium mobilization assay. In respect of its physicochemical properties, compound **40** showed reasonable lipophilicity, good kinetic solubility, and good passive permeability. Substituting with a methyl group on the thiazole ring or on the pyrazole ring increased its potency significantly. Respectively, they obtained compound **41** (ADX88178) ($EC_{50}$ = 13 nM) and compound **42** ($EC_{50}$ = 56 nM). This inspiring result suggested that substitution at the thiazole ring or at the pyrazole ring enabled a favorable interaction with the receptor. ADX88178 was a potent, orally available, and brain-penetrant $mGlu_4$ receptor PAM that showed excellent selectivity against other mGlu receptors. Afterwards, Addex Pharmaceuticals investigated ADX88178's characterizations in rodent models of neuropsychiatric disorders in cooperation with Merck's research laboratory. In 2012, some experimental results[67] showed that ADX88178 reversed haloperidol-induced catalepsy in rats, enhanced the effects of quinpirole in lesioned rats, and also increased the effects of L-DOPA in MitoPark mice. These preclinical tests proved that ADX88178 had a potential therapeutic use for the treatment of Parkinson's disease. In 2014, S. Celanire and co-workers[68] found that ADX88178 dose-dependently reduced the number of buried marbles in the marble burying test, increased open-arm exploration in the elevated plus maze test, and reduced the duration of immobility in the forced-swim test. Thus, these preclinical tests proved that ADX88178 was a useful tool molecule for the treatment of anxiety, depression, psychosis, fear, and obsessive compulsive disorder.

Doller and co-workers[69] synthesized dimethyl derivatives, among which compound **43** was found to show poor potency ($EC_{50}$ > 10 000 nM). They speculated that the two dimethyl groups in compound **43** could not reside on the same side of the molecule because of the steric hindrance, which prevented it from binding with the receptor. So, in order to tether the molecule between the thiazole and pyrazole rings, they designed a series of tricyclic thiazolopyrazoles as potent and selective $mGlu_4$ receptor PAMs. At the beginning, a number of 6-membered-ring derivatives were prepared. Unfortunately, these compounds showed poor potency. In contrast, the 7-membered ring derivatives achieved encouraging results. In this series, compound **44** ($EC_{50}$ = 9 nM) displayed an improved selectivity profile over compound **40**, with good physicochemical properties and excellent brain penetration. We expect the further studies of compound **44** to reveal the value of $mGlu_4$ receptor PAMs (Fig. 17).

**3.1.3. Others.** Using high-throughput screening, Lundbeck's research team[70] found that compound **45** had $mGlu_4$ receptor PAM properties and was selective over other mGlu receptors. But compound **45** suffered from high plasma, suboptimal lipophilicity, and brain non-specific binding. In order to improve its PK properties, they undertook SAR studies and optimized its structure. The SAR studies showed that 1) methyl group additions on the central

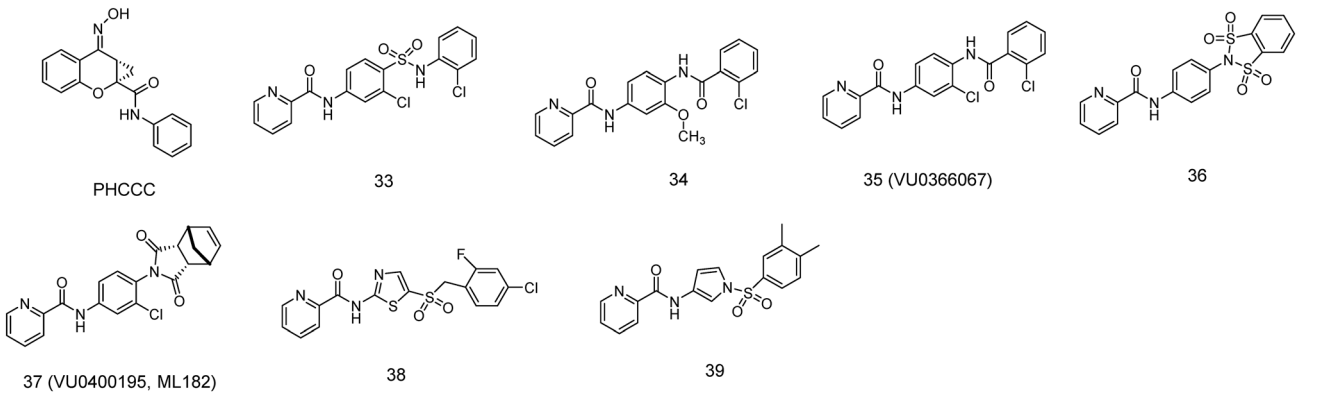



**Fig. 15** The structure of $mGlu_4$ receptor PAMs **33**–**39**.

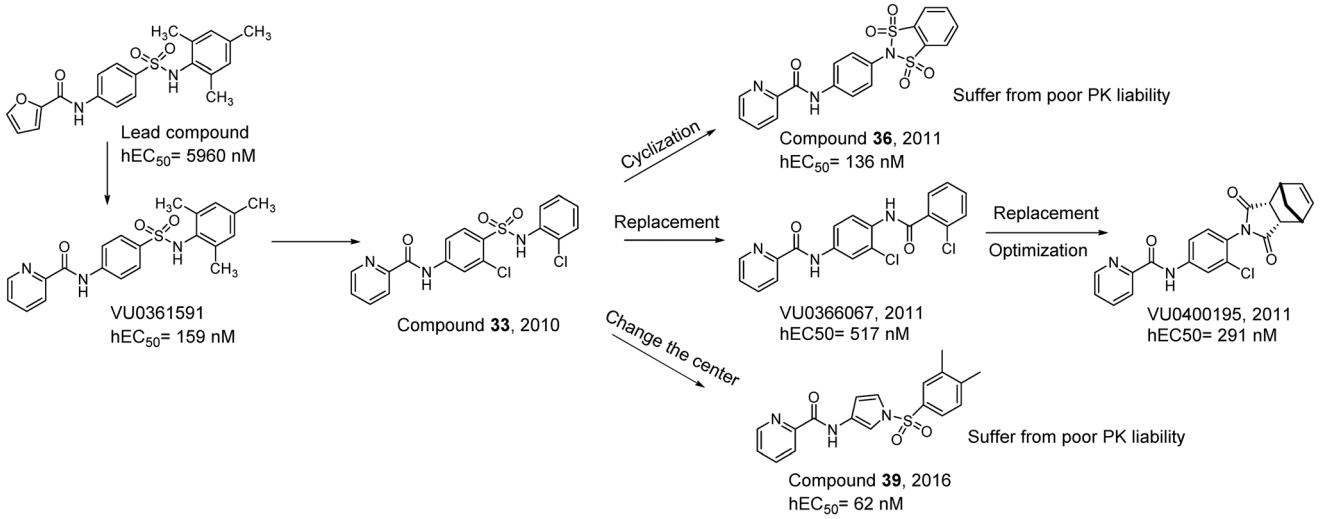



**Fig. 16** Evolution overview of $mGlu_4$ receptor PAMs **33**–**39**.

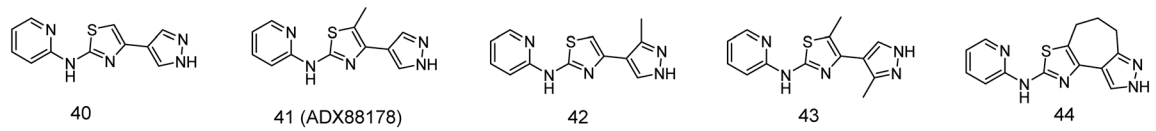



**Fig. 17** The structure of $mGlu_4$ receptor PAMs **40**–**44**.

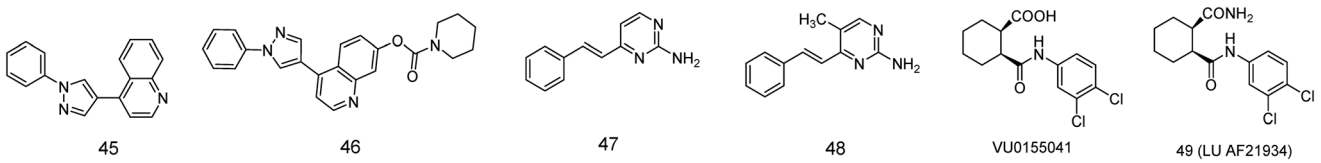



**Fig. 18** The structure of $mGlu_4$ receptor PAMs **45**–**49**.

pyrazole ring were tolerated, and 2) hydrophilic substituents were suitable for the C-7 position of the quinoline ring. Finally, they found that compound **46** showed the most potency, with an $EC_{50}$ value of 190 nM using a calcium mobilization assay.

Gerlach and co-workers[71] found compound **47** displayed $mGlu_4$ receptor PAM properties and wanted to improve its potency and identify compounds suitable for evaluation *in vivo*. SAR studies indicated that the amino group, the olefin, and the phenyl ring were intolerable to substitutions, but a methyl group installed in the C-6 position or in the C-5 position could improve its potency. Finally, they found that compound **48** showed efficacy in a haloperidol-induced catalepsy rat model following oral administration, with an $EC_{50}$ value of 200 nM using an intracellular cAMP assay.

Compound **49** (Lu AF21934)[72] was designed from VU0155041, with an $EC_{50}$ value of 1700 nM using a calcium mobilization assay. Gubellini and co-workers evaluated its properties using some pharmacology techniques. They found that Lu AF21934: 1) inhibited cortico striatal synaptic transmission *in vitro* electrophysiologically, 2) dose-dependently alleviated haloperidol-induced catalepsy, and 3) synergistically alleviated akinesia in a dose-dependent manner combined with sub-threshold doses of L-DOPA. These tests provided an idea that combining L-DOPA and the $mGlu_4$ receptor PAM could reduce efficacious L-DOPA doses in treating Parkinson's disease (Fig. 18).

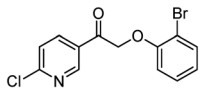



**Fig. 19** The structure of $mGlu_4$ receptor NAMs **50**.

### 3.2. $mGlu_4$ receptor negative allosteric modulators

Only one $mGlu_4$ receptor NAM was found by Vanderbilt University's research team in a high-throughput screening exercise in 2012. Compound **50** (VU0448383)[73] showed an $IC_{50}$ value of 8200 nM using a calcium mobilization assay. SAR

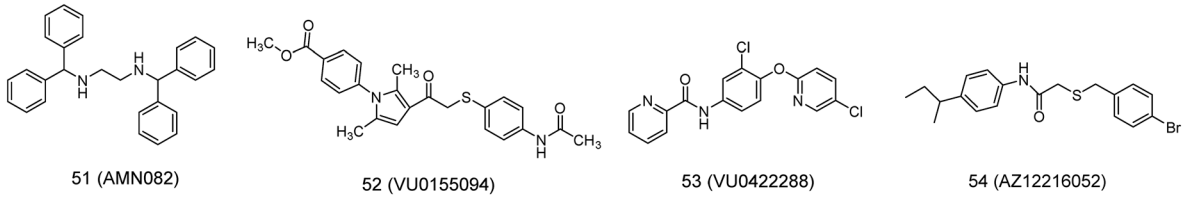



Fig. 20 The structure of other group III glutamate receptor PAMs **51**–**54**.



modifications indicated that only the 4-chloro-3-pyridyl moiety was tolerated in the left-hand moiety, suggesting a shallow binding pocket for that portion of the molecule. Other modifications of the molecule did not support making a breakthrough for its potency (Fig. 19).

### 3.3. Other group III glutamate receptor positive allosteric modulators

Compound **51** (AMN082)[74] was found by Mitsukawa and co-workers as the first orally active and brain-penetrant $mGlu_7$ receptor PAM in 2005. However, in the latest research on its pharmacological characterizations, AMN082 exhibited controversial results.[75] AMN082 had a rapid metabolism to a major metabolite, *N*-benzhydrylethane-1,2-diamine (Met-1), in rat liver microsomes. Rizzo and co-workers demonstrated that Met-1 had potency with the norepinephrine transporter, dopamine transporter, and serotonin transporter, the same as AMN082. After dosage, AMN082 was significantly reduced in the brain, while Met-1 continued to increase. Also, this phenomenon indicated that maybe Met-1 was the true molecule modulating the $mGlu_7$ receptor by additional contributing mechanisms.

In 2014, Niswender and co-workers[76] from Vanderbilt University reported two non-selective group III receptor PAMs, compound **52** (VU0155094) and compound **53** (VU0422288), which showed differential potency at the various group III receptors. VU0155094 showed an $EC_{50}$ value of 3200 nM to the $mGlu_4$ receptor, 1500 nM to the $mGlu_7$ receptor, and 900 nM to the $mGlu_8$ receptor using a calcium mobilization assay. To find a selective $mGlu_7$ or $mGlu_8$ PAM, SAR modifications were undertaken. Although some modifications could improve selectivity to the $mGlu_{7/8}$ and $mGlu_4$ receptors, they did not find any compounds with better potency than that of VU0155094. VU0422288 derived from a chemical optimization program for $mGlu_4$ PAMs, showed an $EC_{50}$ value of 108 nM to the $mGlu_4$ receptor, 146 nM to the $mGlu_7$ receptor, and 125 nM to the $mGlu_8$ receptor. All in all, these compounds were useful tools to probe group III receptor functions, and provided some examples of selective PAMs for the $mGlu_7$ receptor or $mGlu_8$ receptor.

Compound **54** (AZ12216052)[77] was identified by Oregon Health and Science University's research team, with an $EC_{50}$ value of 1000 nM to the $mGlu_8$ receptor using the [$^{35}$S]GTPγS binding assay. It could reduce measures of anxiety in wild-type mice. Through the research on AZ12216052, they found that $mGlu_8$ receptor PAM only affected neurotransmission in the presence of extracellular glutamate, which provided a novel therapeutic target for patients with anxiety disorders accompanying benzodiazepine insensitivity (Fig. 20).

### 3.4. Other group III glutamate receptor negative allosteric modulators

Compound **55** (ADX71743) was a potent, selective, and brain-penetrant $mGlu_7$ receptor NAM, with an $IC_{50}$ value of 63 nM to human and 88 nM to rat $mGlu_7$ receptors using a $Ca^{2+}$ mobilization assay, which was developed through chemical lead optimization by the Addex company. Kalinichev and co-workers investigated its pharmacological characterizations in a series of analytical tests.[78] *In vitro*, ADX71743 was bioavailable after subcutaneous injection administration. Also, *in vivo*, it dose-dependently reduced the number of buried marbles and increased open-arm exploration, which denoted that it had an anxiolytic-like efficacy.

Nakamura and co-workers[79] synthesized and optimized isoxazolopyridone derivatives as novel $mGlu_7$ receptor NAMs based on their previous research.[80] They discovered compound **56** (MMPIP) showed good oral bioavailability, acceptable brain penetrability, and moderate plasma exposure, with an $IC_{50}$ value of 26 nM using a $Ca^{2+}$ mobilization assay. However, according to some further studies on MMPIP, MMPIP might be inactive to the $mGlu_7$ receptor because it showed different effects in distinct cell backgrounds.[81] Hikichi *et al.*[82] also found that MMPIP might impair both non-spatial and spatial recognition function, and was non-selectively inhibited to the central nervous system.

In 2014, Gee and co-workers[83] reported a series of novel selective $mGlu_7$ receptor NAMs. They found that compound **57** (XAP044) exhibited anti-stress, antidepressant, and anxiolytic-like efficacy in a rodent model, with an $IC_{50}$ value of 900 nM using the [$^{35}$S]-GTPγS binding assay (Fig. 21).

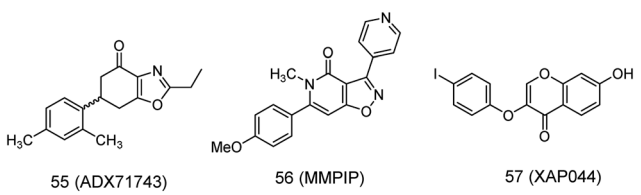



Fig. 21 The structure of other group III glutamate receptor NAMs **55**–**57**.

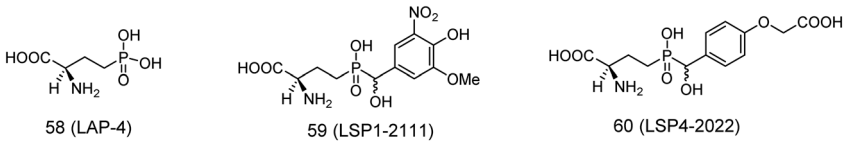



Fig. 22 The structure of $mGlu_{4/6/7/8}$ receptor agonists **58**–**60**.

### 3.5. Non-selective $mGlu_{4/6/7/8}$ receptor agonists

A number of selective $mGlu_{4/6/7/8}$ receptor agonists have been reported. LAP-4 was considered as a starting lead compound toward the discovery of $mGlu_{4/6/7/8}$ receptor agonists. Using a substituted aryl group to replace the hydroxymethylphosphinic acid pharmacophore, F. Acher and co-researcher[84,85] obtained LSP1-2111, a selective $mGlu_4$ receptor agonist. It displayed a selectivity profile of 1-, 25-, and 30-fold *versus* the $mGlu_6$, $mGlu_7$, and $mGlu_8$ receptors, respectively. In 2012, LSP4-2022[86] was found to possess an $EC_{50}$ value of 0.11 nM to the $mGlu_4$ receptor using calcium assays, and an improved selectivity preference of 40-, 100-, and 300-fold over $mGlu_6$, $mGlu_7$, and $mGlu_8$ receptors, respectively (Fig. 22).

## 4. Conclusions

In the past five years, remarkable progress in the area of the mGlu receptors has been made, especially in the group II and group III mGlu receptors. For example, JNJ-46356379 (compound **15**) displayed a remarkable positive allosteric activity and PK profiles to the $mGlu_2$ receptor. On the other hand, ADX88178 (compound **41**) had the most positive activity to the $mGlu_4$ receptor. Meanwhile, some $mGlu_{2/3}$ receptor agonists, such as LY2812223 and compound **31**, showed a fascinating characteristic to $mGlu_{2/3}$ receptors. In clinical studies, JNJ-40411813 (compound **10**), a selective $mGlu_2$ receptor PAM, was halted at phase II study in depressive patients because of its poor efficacy. LY2140023, an $mGlu_{2/3}$ receptor agonist developed by Eli Lilly, advanced into a phase III trial in patients with schizophrenia. Other $mGlu_{2/3}$ receptor NAMs and RO4995819 also progressed into a phase II trial to cure depressive disorder. It is worth mentioning that each laboratory has its own analysis assay for potency, which may actually hinder the development of mGlu receptor modulators.

In view of our enumeration of the typical structures of various mGlu receptor allosteric modulators and agonists/antagonists, it is believed that more and more mGlu receptor allosteric modulators and agonists/antagonists as neurological disease agents will be discovered and applied in the near future.

## Acknowledgements

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